

CLAIMS

1. Complex between at least one (negatively charged) nucleic acid and at least one positively charged polymeric conjugate, the bond between the nucleic acid and the polymeric conjugate being electrostatic in nature and the polymeric conjugate containing a polymer formed from monomer units carrying free NH_3^+ functions, and being such that:

- the free NH_3^+ functions of the abovementioned monomer units are substituted in a ratio of at least 10%, advantageously about 15% to about 45%, in particular 35%, this ratio being determined, for example, by nuclear magnetic resonance, by residues which can be protonated in a weakly acid medium causing destabilization of cell membranes, in particular the membrane of endocytosis vesicles, and/or of endosomes in a weakly acid medium,

- the abovementioned residues also having the following properties:

. they carry a functional group which enables them to be bonded to the abovementioned polymer,

. they are not active with respect to the recognition signal recognized by a cell membrane receptor,

. they can carry at least one free NH_3^+ function,

- it being possible for the free NH_3^+ functions of the abovementioned monomer units also to be substituted by non-charged residues causing a reduction in the positive charges with respect to the same unsubstituted polymeric conjugate, facilitating salting out of the nucleic acid in the course of dissociation of the complex,

- the abovementioned non-charged residues also having the following properties:

. they carry at least one hydroxyl group,

. they are not active with respect to the recognition signal recognized by a cell membrane receptor,

- molecules constituting a recognition signal recognized by a cell membrane receptor optionally being present:

. by substitution of some of the free NH_3^+ functions of the abovementioned monomer units (for example $\epsilon\text{-NH}_3^+$ of lysine), or

. on some of the abovementioned non-charged residues causing a reduction in the charge (for example gluconyl), in particular on the hydroxyl groups of the abovementioned non-charged residues, or

. on some of the abovementioned residues causing a destabilization of cell membranes (for example acetylimidazole), or

. by substitution of the optional free NH_3^+ function of the abovementioned residues causing a destabilization of cell membranes (for example histidine),

5 with the proviso that all the free NH_3^+ functions make up at least 30% of the number of monomer units of the polymeric skeleton of the abovementioned polymeric conjugate.

2. Complex between at least one (negatively charged) nucleic acid and at least one positively charged polymeric conjugate, the bond between the nucleic acid and the polymeric
10 conjugate being electrostatic in nature and the polymeric conjugate containing a polymer formed from monomer units carrying free NH_3^+ functions, and being such that:

- the free NH_3^+ functions of the abovementioned monomer units are substituted in a ratio of at least 10%, advantageously about 15% to about 45%, in particular 35%, this ratio being determined, for example, by nuclear magnetic resonance, by residues which can be
15 protonated in a weakly acid medium causing destabilization of cell membranes, in particular the membrane of endocytosis vesicles and/or of endosomes, in a weakly acid medium,

- the abovementioned residues also having the following properties:

. they are bases of which the pK in an aqueous medium is less than 8, such that a proportion greater than 50% of these bases bonded to a cationic polymer is not protonated in a neutral medium of pH 7.4,

. they carry a functional group which enables them to be bonded to the abovementioned polymer,

. they are not active with respect to the recognition signal recognized by a cell membrane receptor,

. they can carry at least one free NH_3^+ function,

- it being possible for the free NH_3^+ functions of the abovementioned monomer units also to be substituted by non-charged residues causing a reduction in the positive charges with respect to the same unsubstituted polymeric conjugate, facilitating salting out of the nucleic acid in the course of dissociation of the complex,

30 - the abovementioned non-charged residues also having the following properties:

. they carry at least one hydroxyl group,

. they are not active with respect to the recognition signal recognized by a cell

membrane receptor,

- molecules constituting a recognition signal recognized by a cell membrane receptor optionally being present:

- . by substitution of some of the free NH_3^+ functions of the abovementioned monomer units (for example $\epsilon\text{-NH}_3^+$ of lysine), or
 - . on some of the abovementioned non-charged residues causing a reduction in the charge (for example gluconyl), and in particular on the hydroxyl groups of the abovementioned non-charged residues causing a reduction in charge, or
 - . on some of the abovementioned residues causing a destabilization of cell membranes (for example acetylimidazole), or
 - . by substitution of the optional free NH_3^+ function of the abovementioned residues causing a destabilization of cell membranes (for example histidine),
- with the proviso that all the free NH_3^+ functions make up at least 30% of the number of monomer units of the polymeric skeleton of the abovementioned polymeric conjugate.

3. Complex between at least one (negatively charged) nucleic acid and at least one positively charged polymeric conjugate, the bond between the nucleic acid and the polymeric conjugate being electrostatic in nature and the polymeric conjugate containing a polymer formed from monomer units carrying free NH_3^+ functions, and being such that:

- the free NH_3^+ functions of the abovementioned monomer units are substituted in a ratio of at least 10%, advantageously about 15% to about 45%, in particular 35%, this ratio being determined, for example, by nuclear magnetic resonance, by residues which can be protonated in a weakly acid medium causing destabilization of cell membranes, in particular the membrane of endocytosis vesicles, in a weakly acid medium,

- the abovementioned residues also having the following properties:

- they belong to the family of compounds which carry an imidazole nucleus,
- . they belong to the family of quinolines,
- . they belong to the family of pterines,
- . they belong to the family of pyridines,
- . the abovementioned residues carry a functional group which enables them to be bonded to the abovementioned polymer,
- . they can carry at least one free NH_3^+ function,

. they are not active with respect to the recognition signal recognized by a cell membrane receptor,

- it being possible for the free NH_3^+ functions of the abovementioned monomer units also to be substituted by at least one molecule which constitutes a recognition signal recognized by a cell membrane receptor, and/or by non-charged residues causing a reduction in the positive charges with respect to the same unsubstituted polymeric conjugate, facilitating salting out of the nucleic acid in the course of dissociation of the complex, with the proviso that all the abovementioned residues contain at least 30% of free NH_3^+ functions,

- it being possible for the free NH_3^+ functions of the abovementioned monomer units also to be substituted by at least one molecule which constitutes a recognition signal recognized by a cell membrane receptor, and/or by non-charged residues causing a reduction in the positive charges with respect to the same unsubstituted polymeric conjugate, facilitating salting out of the nucleic acid by dissociation of the complex,

- the abovementioned non-charged residues also having the following properties:

. they carry at least one hydroxyl group,

. they are not active with respect to the recognition signal recognized by a cell membrane receptor,

- molecules constituting a recognition signal recognized by a cell membrane receptor optionally being present:

. by substitution of some of the free NH_3^+ functions of the abovementioned monomer units (for example $\epsilon\text{-NH}_3^+$ of lysine), or

. on some of the abovementioned non-charged residues causing a reduction in the charge (for example gluconyl), and in particular on the hydroxyl groups of the abovementioned non-charged residues causing a reduction in charge, or

. on some of the abovementioned residues causing a destabilization of cell membranes (for example acetylimidazole), or

. by substitution of the optional free NH_3^+ function of the abovementioned residues causing a destabilization of cell membranes (for example histidine),

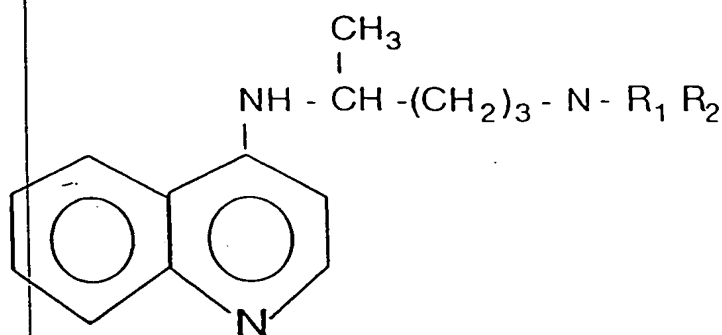
with the proviso that all the free NH_3^+ functions make up at least 30% of the number of monomer units of the polymeric skeleton of the abovementioned polymeric conjugate.

4. Complex according to one of claims 1 to 3, in which the residues causing

destabilization of cell membranes in a weakly acid medium are

- alkyimidazoles in which the alkyl radical contains 1 to 10, in particular 2 to 6 carbon atoms, and in which only one of the nitrogen atoms of the imidazole nucleus is substituted,

- or quinolines of the formula:



in which R_1 represents H and R_2 represents $(CH_2)_n-CO_2-H$, n being an integer varying from 1 to 10, and preferably having a value of 1 to 3.

claim 1
5. Complex according to ~~any one of claims 1 to 3,~~

in which the residues causing destabilization of cell membranes are chosen from: histidine, 4-carboxymethyl-imidazole, 3-(1-methyl-imidazol-4-yl)-alanine, 3-(3-methyl-imidazol-4-yl)-alanine, 2-carboxy-imidazole, histamine, 3-(imidazol-4-yl)-L-lactic acid, 2-(1-methyl-imidazol-4-yl)ethylamine, 2-(3-methyl-imidazol-4-yl)ethylamine, β -alanyl-histidine- (carnosine), 7-chloro-4-(amino-1-methylbutylamino)-quinoline, N^4 -(7-chloro-4-quinolinyl)-1,4-pentanediamine, 8-(4-amino-1-methylbutylamino)-6-methoxyquinoline (primaquine), N^4 -(6-methoxy-8-quinolinyl)-1,4-pentanediamine, quininic acid, quinolinecarboxylic acid, pteric acid, nicotinic acid and quinolinic acid,

and in which

- the optional free NH_3^+ function of the abovementioned residues (for example histidine) can also be substituted by a molecule which constitutes a recognition signal recognized by a cell membrane receptor,

with the proviso that all the free NH_3^+ functions make up at least 30% of the number of

monomer units of the polymeric skeleton of the abovementioned polymeric conjugate.

6. Complex according to ^{claim 1} ~~one of claims 1 to 5~~ between at least one (negatively charged) nucleic acid and at least one positively charged polymeric conjugate, the bond between the nucleic acid and the polymeric conjugate being electrostatic in nature and the polymeric conjugate containing a polymer formed from monomer units carrying free NH_3^+ functions, in particular residues of lysine or ornithine, and being such that:

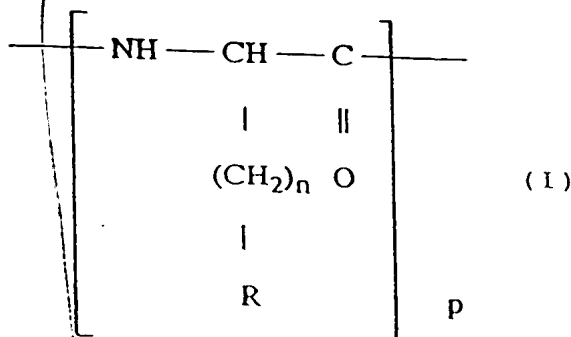
- the free NH_3^+ functions of the abovementioned monomer units are substituted in a ratio of at least 10%, advantageously about 15% to about 45%, in particular 35%, by residues causing a destabilization of cell membranes in a weakly acid medium,

- the abovementioned residues also having the following properties:

- . they carry an imidazole nucleus,
- . they can carry at least one free NH_3^+ function,
- . they are not active with respect to the recognition signal,

- the remaining free NH_3^+ functions of the abovementioned monomer units also being substituted to the extent of about 1% to about 60% by a molecule which constitutes a recognition signal recognized by a cell membrane receptor, this recognition signal having a molecular weight of less than 5,000, and it being possible for this recognition signal to be present in an amount of one molecule for about 200 units of polymeric conjugate or about 60 molecules for about 200 units of polymeric conjugate, with the proviso that all the free NH_3^+ functions make up at least 30% of the number of monomer units of the polymeric skeleton of the abovementioned polymeric conjugate.

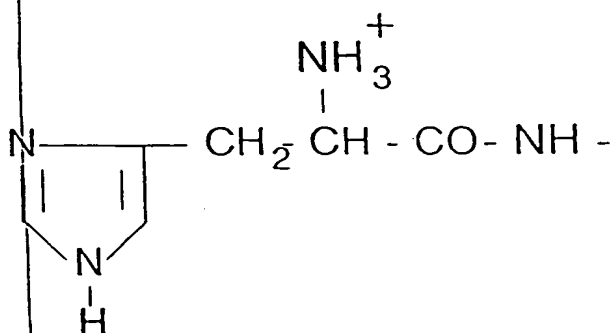
7. Complex according to ^{claim 1} ~~one of claims 1 to 3~~, in which the polymer contains a polymeric grouping of the following formula (I):



in which:

- p is an integer varying from 15 to 900, preferably 100 to 300,
- n is an integer varying from 1 to 6, and preferably has the value 4,
- this polymeric grouping contains radicals R among which:

10% to 45% of the number of radicals R representing a residue carrying an imidazole nucleus and optionally a free NH_3^+ function, in particular a histidyl residue, it being possible for R to be represented by the formula:



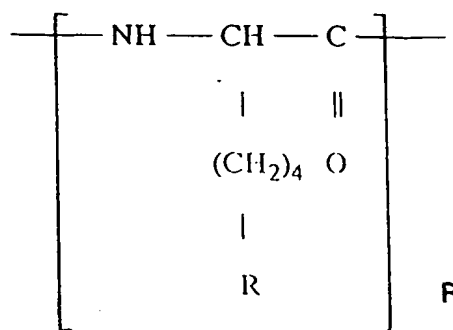
it being possible for the optional NH_3^+ function of the abovementioned residues also to be substituted by a molecule which constitutes a recognition signal,

10% to 90% of the number of radicals R representing free α -amino NH_3^+ and optionally being substituted to the extent of 0 to 50% by a molecule which constitutes a recognition signal, in particular to the extent of 0 to 60, advantageously 1 molecule for about 200 units, or to the extent of 2 to 100, advantageously 50 molecules for about 200 units, and/or

it also being possible for R to be made up to the extent of 0 to 45% of a group $\text{NH-CO-(CHOH)}_m\text{-R}_1$, in particular a dihydroxypropionylamido, erythrionylamido, threonylamido, ribonylamido, arabinylamido, xylonylamido, lyxonylamido, gluconylamido, galactonylamido, mannonylamido, glycoheptonylamido or glycooctonylamido radical, m is an integer from 2 to 15, preferably 2 to 7, R_1 represents H or an alkyl radical having 1 to 15 carbon atoms, in particular CH_3 , it being possible for these radicals to be substituted by a molecule which constitutes a recognition signal, with the proviso that all the free NH_3^+ functions make up at least 30% of the number of monomer units of the polymeric skeleton of

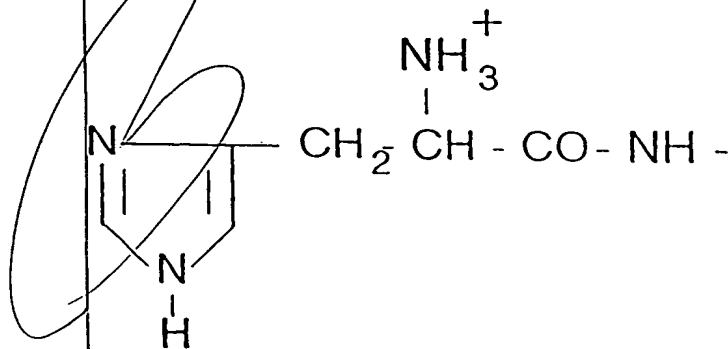
the abovementioned polymeric conjugate.

8. Complex according to claim 4, in which the polymer comprises a polymeric grouping of the following formula (II):



in which:

- p has the meanings indicated in claim 4,
- 10% to 45% of the number of radicals R represent a residue carrying an imidazole nucleus and optionally a free NH_3^+ function, in particular a histidyl residue, it being possible for R to be represented by the formula



it being possible for the NH_3^+ functions of the abovementioned residues also to be substituted by a molecule which constitutes a recognition signal,

- the remainder of the radicals, that is to say 30% to 90% of the number of radicals R,

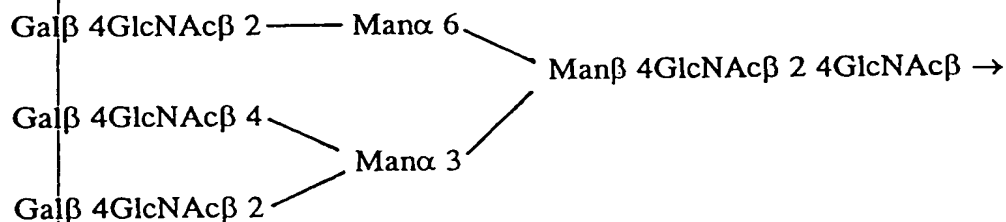
representing ω -amino NH_3^+ , and it being possible for 0 to 45% of the radicals R to be substituted by a molecule which constitutes a recognition signal recognized by a cell membrane receptor,

with the proviso that all the free NH_3^+ functions make up at least 30% of the number of monomer units of the polymeric skeleton of the abovementioned polymeric conjugate.

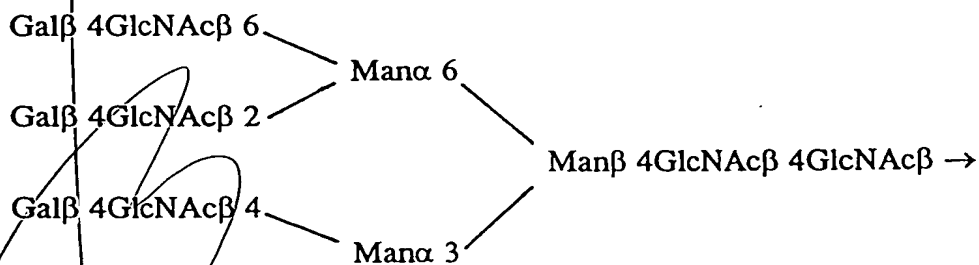
election a 9. Complex according to ^{claim 1} ~~one of claims 1 to 5~~, characterized in that the recognition signal is chosen from:

A) - simple or complex osides recognized by membrane lectins and chosen from:

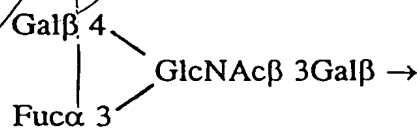
10 a. Asialo-oligoside of the type of triantennar lactosamine: asialoglycoprotein receptor



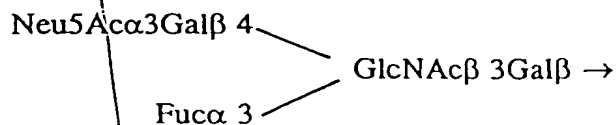
b. Asialo-oligoside of the type of tetraantennar lactosamine: asialoglycoprotein receptor



c. Lewis x: LECAM 2/3

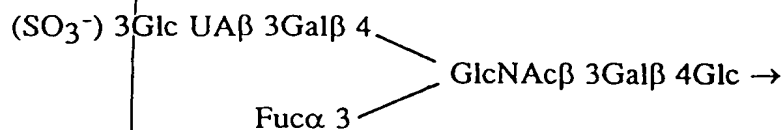


d. Lewis x sialyl: LECAM 3/2

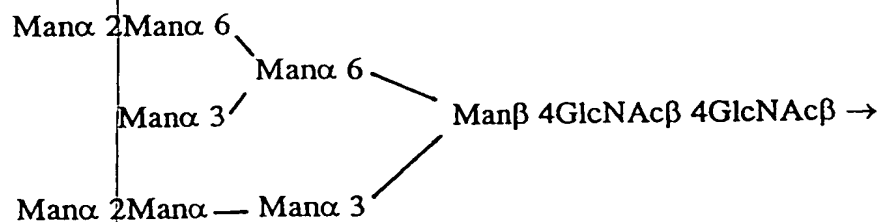


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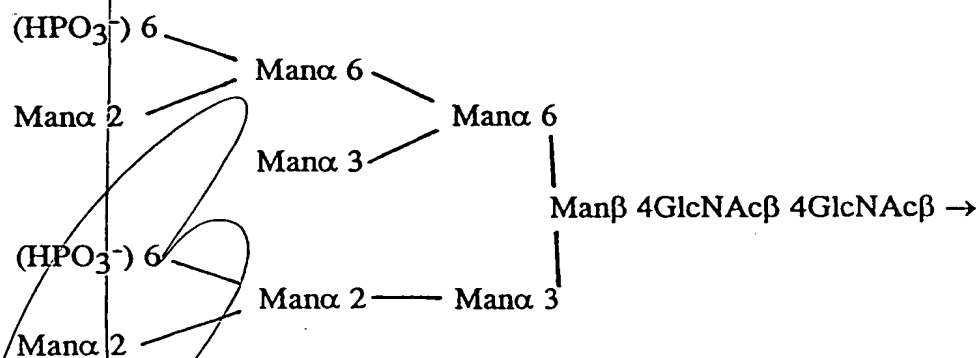
e. Sulphated Lewis x derivative (HNK1): LECAM 1



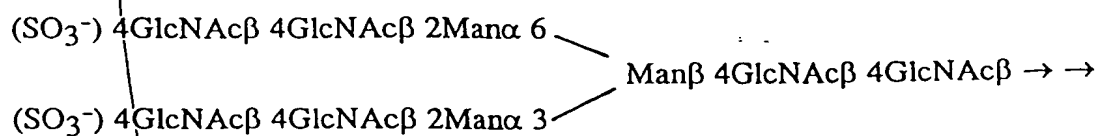
f. Oligomannoside: mannose receptor



g. Phosphorylated oligomannoside: mannose 6-phosphate receptor



h. Oligosaccharide of the type of sulphated lactosamine: sulphated GalNAc 4 receptor



B) Peptides

a) anti-inflammatory peptides or certain of their fragments recognized by receptors of the vascular wall, such as

- vasodilator intestinal polypeptide (VIP)

HSDAVFTDNYTRLRKQMAVKKYLNSILN-NH₂

- atrial natriuretic polypeptide (ANP)

SLRRSSCFGGRMDRIGAQSGLGCNSFRY

- lipocortin

HDMNKVLDL

- bradykinin

RPPGFSPFR;

b) ligand peptides of integrins, such as peptides containing the sequence RGD, fibronectin ligand;

c) chemiotactic factors, such as formyl-peptides and their antagonists: FMLP, (N-formyl-Met-Leu-Phe);

d) peptide hormones, such as α -MSH: Ac-SYSMEHFRWGKPV-NH₂ and their antagonists.

C) Natural metabolites, such as:

- biotin,

- carnitine,

- tetrahydrofolate and folic acid, which can be both a recognition signal with respect to certain cells having suitable receptors and a destabilizer of cell membranes.

a 10. Complex according to ^{claim 1} ~~one of claims 1 to 6,~~ characterized in that the nucleic acid can be chosen from:

a) marker genes, such as

- genes containing luciferase,

- green protein of the jellyfish *Aequorea victoria*,

- genes containing β -galactosidase,

- genes containing chloramphenicol acetyltransferase,

- genes which confer resistance to an antibiotic, such as hygromycin, neomycin etc....;

b) genes with a therapeutic purpose, such as

- receptors of lipoproteins of low-density, which are deficient in cases of hypercholesterolaemia,

- coagulation factors: factors VIII and IX,

- phenylalanine hydroxylase (phenylketonuria),

- adenosine deaminase (ADA immunodeficiency),

- lysosomal enzymes, such as β -glucosidase in the case of Gaucher's disease,

- dystrophin and minidistrophin (myopathy),

- tyrosine hydroxylase (Parkinson),

- neurone growth factors (Alzheimer),

- CFTR cystic fibrosis transmembrane conductance regulator (cystic fibrosis),

- alpha-1-antitrypsin,

- cytokines (interleukins, TNF tumour necrosing factor),

- thymidine kinase of the Herpes simplex virus,

- proteins of MHC, major histocompatibility complex, in particular HLA-B7,

- cytosine deaminase,

- genes which code for sense and antisense RNAs,

- genes which code for ribozymes,

c) genes for the purpose of vaccines

- genes which code for viral antigens (vaccination), for example: the gene which codes for the nucleoprotein of the influenza virus.

11. Complex according to ^{claim} one of claims 1 to 7, in which:

- the polymer, in particular polylysine, has a degree of polymerization of about 15 to about 900, preferably 200,

- the free NH_3^+ functions of the lysine units being substituted in a ratio of 35% by histidyl residues and optionally by a molecule which constitutes a recognition signal for 1 to 50 residues of lysine, where the said signal molecule has an affinity of at least 10^5 l mole^{-1} with respect to the receptor of the cell which the complex is to target, or optionally by 20 to 100 molecules of recognition signal for 200 lysine residues, where the said signal molecule

has an affinity of less than 10^5 l mole^{-1} with respect to the said receptor,

- the nucleic acid has a molecular weight of about 10^6 to about 10^8 , in particular $3 \cdot 10^6$ to $30 \cdot 10^6$,

- the ratio between the average number of base pairs of the nucleic acid per molecule of monomer unit, in particular lysine, is about 0.2 to about 6, preferably about 0.4 to about 0.6.

12. Positively charged polymeric conjugate containing units carrying free NH_3^+ functions, and being such that:

- the free NH_3^+ functions of the abovementioned monomer units are substituted in a ratio of at least 10%, advantageously about 15% to about 45%, in particular 35%, this ratio being determined, for example, by nuclear magnetic resonance, by residues causing a destabilization of cell membranes, in particular the membrane of endocytosis vesicles, in a weakly acid medium,

- the abovementioned residues also having the following properties:

- . they carry a functional group which enables them to be bonded to the abovementioned polymer,
- . they are not active with respect to the recognition signal recognized by a cell membrane receptor,
- . they can carry at least one free NH_3^+ function,

- it being possible for the free NH_3^+ functions of the abovementioned monomer units also to be substituted by non-charged residues causing a reduction in the positive charges with respect to the same unsubstituted polymeric conjugate, facilitating salting out of the nucleic acid by dissociation of the complex,

- the abovementioned non-charged residues also having the following properties:

- . they carry at least one hydroxyl group,
- . they are not active with respect to the recognition signal recognized by a cell membrane receptor,
- . it being possible for the hydroxyl groups of the abovementioned non-charged residues to be substituted by at least one molecule which constitutes a recognition signal recognized by a cell membrane receptor,

- molecules constituting a recognition signal recognized by a cell membrane receptor optionally being present:

- . by substitution of some of the free NH_3^+ functions of the abovementioned monomer units (for example $\epsilon\text{-NH}_3^+$ of lysines), or
 - . on some of the abovementioned non-charged residues causing a reduction in the charge (for example gluconyl), and in particular on the hydroxyl groups of the abovementioned non-charged residues causing a reduction in charge, or
 - . on some of the abovementioned residues causing a destabilization of cell membranes (for example acetylimidazole), or
 - . by substitution of the optional free NH_3^+ function of the abovementioned residues causing a destabilization of cell membranes (for example histidine),
- with the proviso that all the free NH_3^+ functions make up at least 30% of the number of monomer units of the polymeric skeleton of the abovementioned polymeric conjugate.

~~13. Polymeric conjugate according to claim 12 and as defined according to one of claims 2 or 3, or containing a polymeric grouping of the formula according to one of claims 4 or 5,~~

14. Use of a complex according to one of claims 1 to 11 or a conjugate according to one of claims 12 or 13 for the *in vitro*, *ex vivo* or *in vivo* transfection of cells with the aid of a gene, in particular those defined in claim 6.

~~15. Use of a complex or a conjugate according to claim 11, characterized in that the cells are chosen from:~~

- cells of haematopoietic strains;
- dendritic cells;
- liver cells;
- skeletal muscle cells;
- skin cells:
- . fibroblasts,
- . keratinocytes,
- . dendritic cells,

- . melanocytes;
- cells of the vascular walls;
- . endothelial;
- . smooth muscle;
- epithelial cells of the respiratory tract;
- cells of the central nervous system;

a' - cancerous cells; and

a - cells of the immune system, such as lymphocytes, macrophages, NK cells

etc.

16. Method of *in vitro* or *ex vivo* transfection, characterized in that a complex according to claim 1 ~~any one of claims 1 to 11~~ is brought into contact with a medium containing cells to be transfected under conditions such that there is:

- passage of the complex from the medium into the cytoplasm of the cells,
- salting out of the nucleic acid involved in the abovementioned complex in the cytosol and/or the nucleus of the cells,
- transcription and expression of the nucleic acid in the transfected cells,
- expression of the protein corresponding to the transfected gene.

17. Pharmaceutical composition, characterized in that it comprises, as the active substance, at least one of the complexes according to claim 1 ~~any one of claims 1 to 11~~, or at least one of the conjugates according to claim 12 ~~one of claims 12 or 13~~, in combination with a pharmaceutically acceptable vehicle.

18. Use of a complex according to one of claims 1 to 11 or a conjugate according to one of claims 12 or 13 for the preparation of a medicament intended, for example, for treatment of congenital or acquired metabolic deficiency, or treatment of tumours, or for the preparation of a vaccine, for example a vaccine against influenza.

19. Set or kit comprising:

- a - a polymeric conjugate according to claim 12 with a ~~one of claims 12 or 13~~, such as polylysine substituted by a residue causing a destabilization of cell membranes in a weakly acid

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medium, this polymeric conjugate being capable of optionally carrying a recognition signal, which is or is not bonded beforehand to the abovementioned polymeric conjugate, the said recognition signal being a function of the cell to be targeted,

- optionally a plasmid containing at least one gene to be transferred, and optionally the
- 5 system for regulation of the expression of the abovementioned gene,
- reagents which allow optional bonding of the recognition signal on to the abovementioned polymeric conjugate,
- reagents which allow the formation of a complex according to ^{claim 1} ~~one of claims I to II~~,
- a or between the polymeric conjugate and the gene to be transferred, or between the polymeric
- 10 conjugate and a plasmid containing the gene to be transferred,
- reagents which allow transfection of the cell by the abovementioned complex.

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